Use Of Midori Green For DNA Content Analysis By Flow Cytometry In Budding Yeast (Saccharomyces Cerevisiae)

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Abstract

For flow cytometry applications, chromosomal DNA of budding yeast cells has been stained routinely with propidium iodide but SYBR® Green tends to yield superior results. Use of the recently introduced Midori Green results in staining of equal quality at significantly lower costs.

INTRODUCTION

Flow cytometry is the method of choice for assaying DNA content distribution within a large number of cells during the analysis of cell cycle stage. The small DNA content of haploid cells of yeasts such as budding yeast (Saccharomyces cerevisiae) complicates such analysis but satisfying profiles can be obtained with propidium iodide (PI) staining (1). Several investigators have reported superior results with SYBR Green I, a notion I have confirmed. However, large-scale routine use of SYBR Green may add a significant cost factor. Using an established protocol (2) and current pricing, I estimate expenses of at least $ 300 per 1,000 samples. Another green dye, Midori Green has recently been made commercially available in the USA for non-toxic staining of DNA in agarose gels. I explored if Midori Green can be used for flow cytometry and provide an alternative to SYBR Green.

MATERIAL AND METHODS

The following protocol, adapted from (2), was used with logarithmic-phase haploid cells of Saccharomyces cerevisiae (strain BY4741, obtained from Open Biosystems).

1. Samples of at least 5x106 yeast cells were collected, washed with water, resuspended in 100% ethanol for fixation, typically for 24 h at 4 °C. I have reduced the period of fixation to 10 min or less without loss of signal or peak resolution.
2. Cells were spun down, washed with 1 ml of sterile deionized water, resuspended in 0.5 ml 50 mM sodium citrate (pH 7.0).
3. 4 µl of 10 mg/ml RNase A (in 100 mM NaCl/10 mM TRIS-HCl/1 mM EDTA, pH 8.0 [STE], previously boiled to inactivate DNase) was added and samples were incubated for 1-2 h at 50°C.
4. 12 µl of 10 mg/ml proteinase K (in water) was added and incubation continued for 1 h at 50 °C.
5. Midori Green Advance (Bulldog Bio Inc, Portsmouth, NH) was diluted 1:20 in TE/0.25% Triton X100 and 4 µl was added per cell sample.
6. 0.5 ml of 50 mM sodium citrate was added. Sodium citrate solution may be supplemented with 16 µg/ml PI if one wishes to analyze Midori Green (on FL1 channel) and PI fluorescence (FL3 channel) simultaneously.
7. Samples were incubated overnight at 4°C, transferred to plastic tubes compatible with the flow cytometer used, sonicated and analyzed.

RESULTS AND DISCUSSION

Figure 1

Flow cytometry profiles of aliquots of the same sample of logarithmic-phase haploid wild-type yeast cells, stained with SYBR Green (left) or Midori Green (right). Using ModFit LT TM software, the percentages of G1/S/G2-M cells were determined as indicated. The sample was taken during a release-from-synchronization experiment.

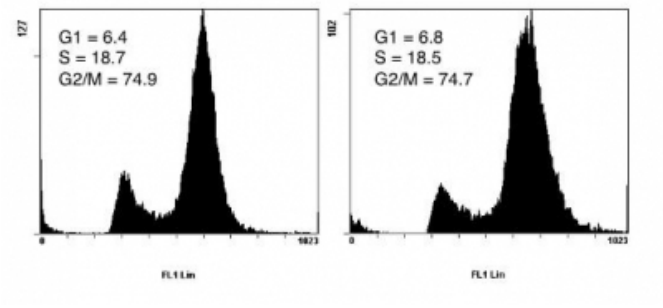

Figure 1 shows aliquots of the same cell sample, stained in parallel with SYBR Green and Midori Green. It is apparent that signal strength, resolution and peak width is satisfactory in both cases and quite comparable. Furthermore, quantifying the G1/S/G2-M cell portions with commercial software yielded identical numbers (Fig. 1). Since less dye is used, use of Midori Green will be significantly cheaper than SYBR Green, reducing the price of analysis of 1,000 samples from $ 300 to $ 40.

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